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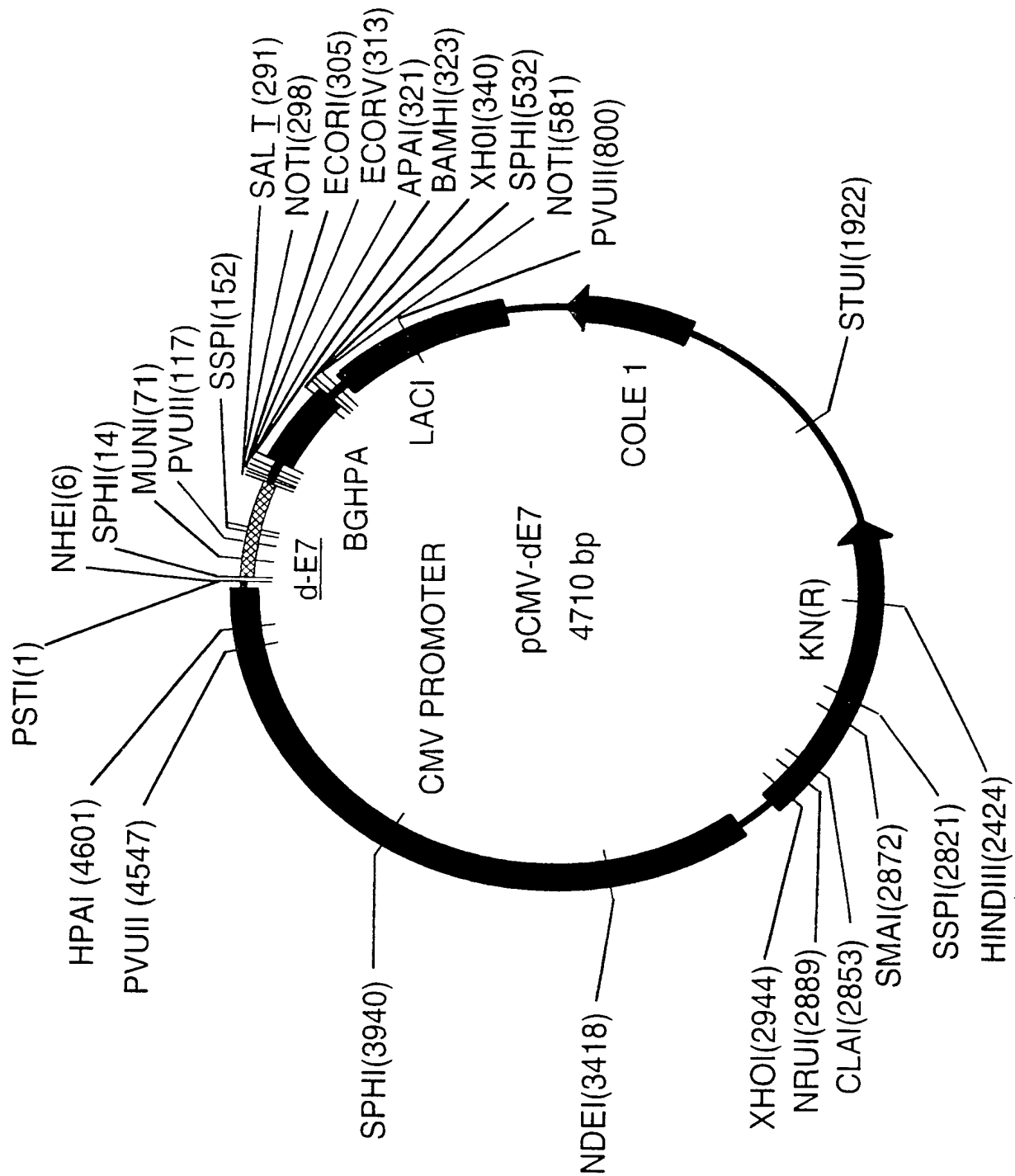


FIG.1A

FIG.1B

Nucleotide Sequence of PCR Amplified dE7 from HPV-16

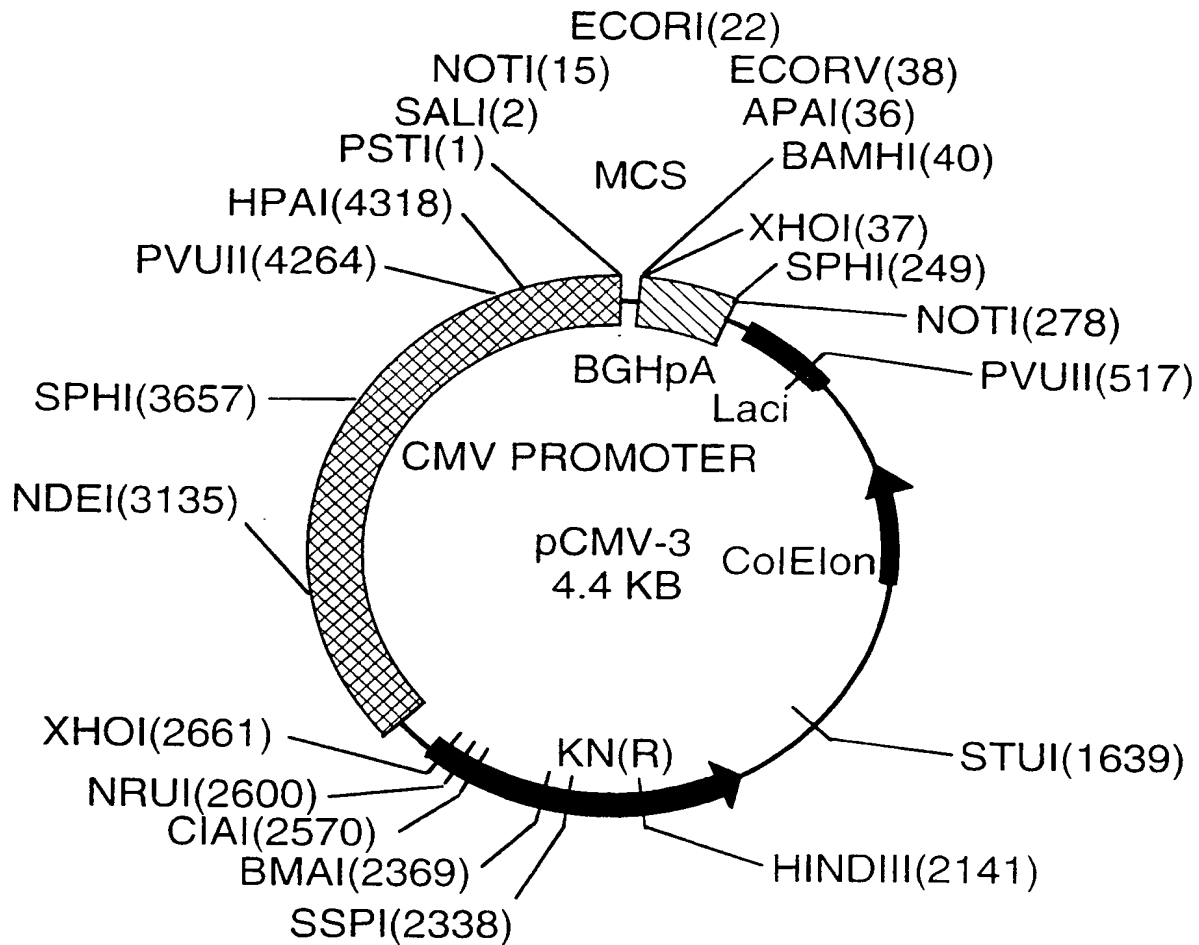
5' PCR Primer →

CTGCAGCAGG	CTAGCATGCA	TCGAGATACA	CCTACATTGC	ATCAATATAT	GTTAGATTTG
CAACCAGAGA	CAACTCAATT	GAATGACAGC	TCAGAGGAGG	AGGATGAAAT	AGATGGTCCA
GCTGGACAAG	CAGAACCGGA	CAGAGCCCAT	TACAATATTG	TAACTTTTG	TTGCAAGTGT
GACTCTACGC	TTTCGGTTGIG	CGTACAAAGC	ACACACGTAG	ACATTCTGAC	TTTTCGAAGAC
CTGTTAATGG	GCACACTAGG	AATTGIGTGC	CCCATCTGTT	CTCAGAAACC	ATAAGTTCGAC

← 3' PCR PRIMER

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pCMV-3 vector containing CMV promoter, Bovine Growth Hormone polyA and Kanamycin resistance

FIG.2

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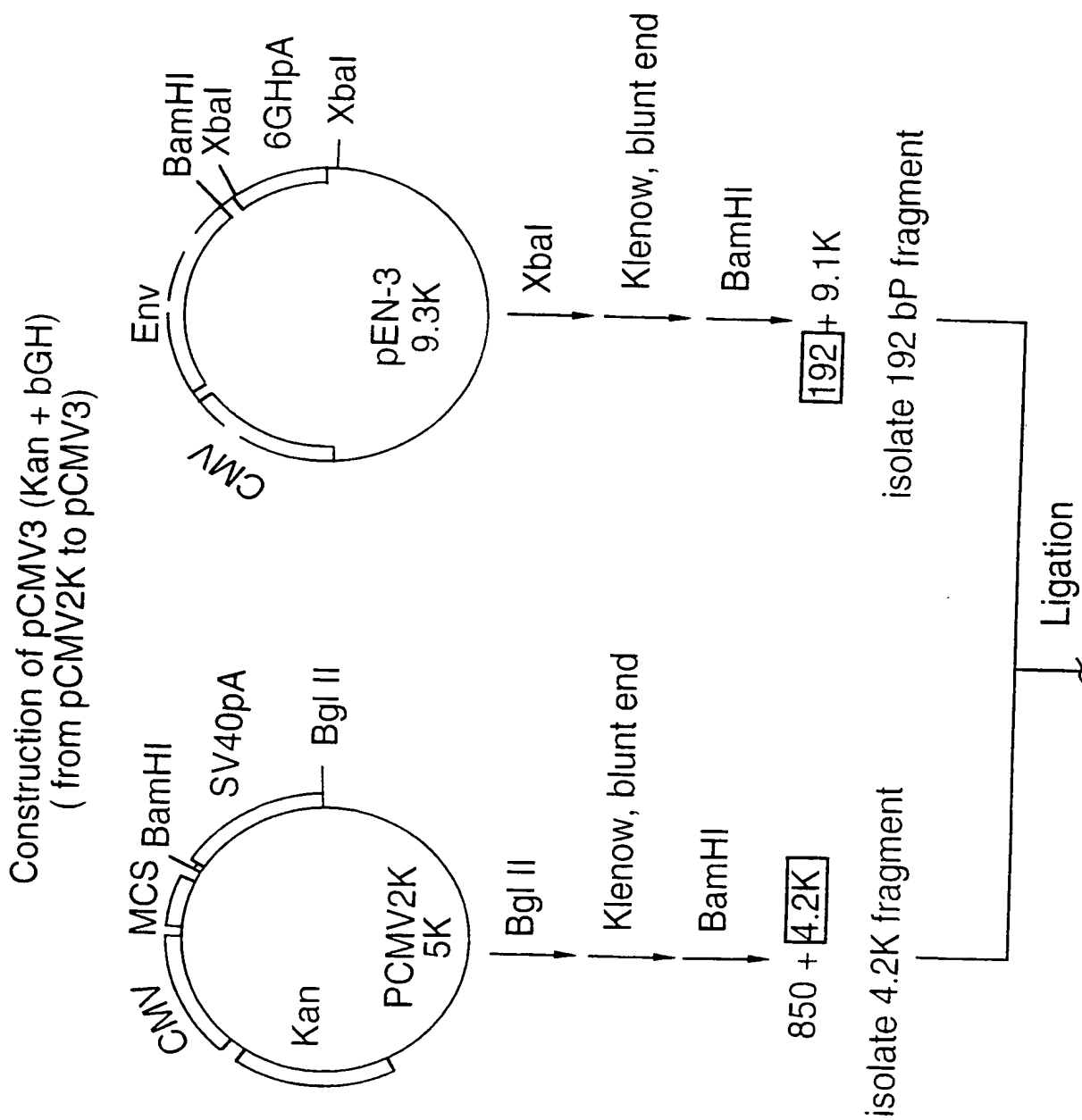
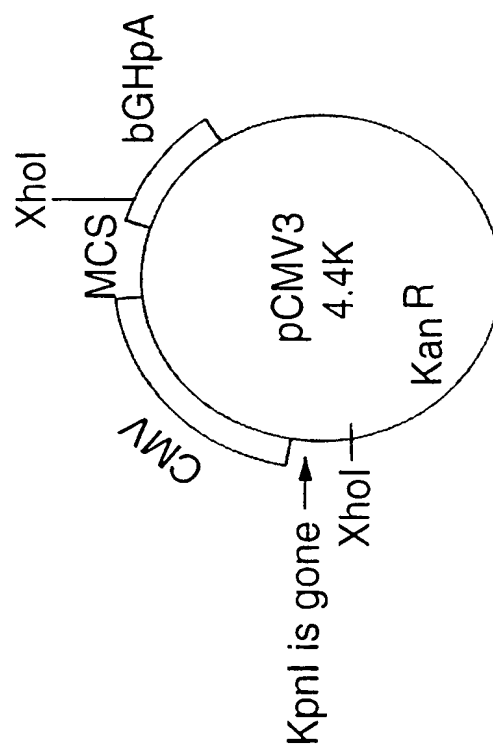


FIG.3A

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Transformation, use Kan^R plates
pCMV3



pEN - 3: cloning of bGHpA into
pEN-1
pCMV2K is pCMV2 Amp^R
replaced with Kan^R
pCMV2: pEN-1
with cmv insert
oligo linker

* see pEN-1 diagram (Fig.4)

FIG.3B

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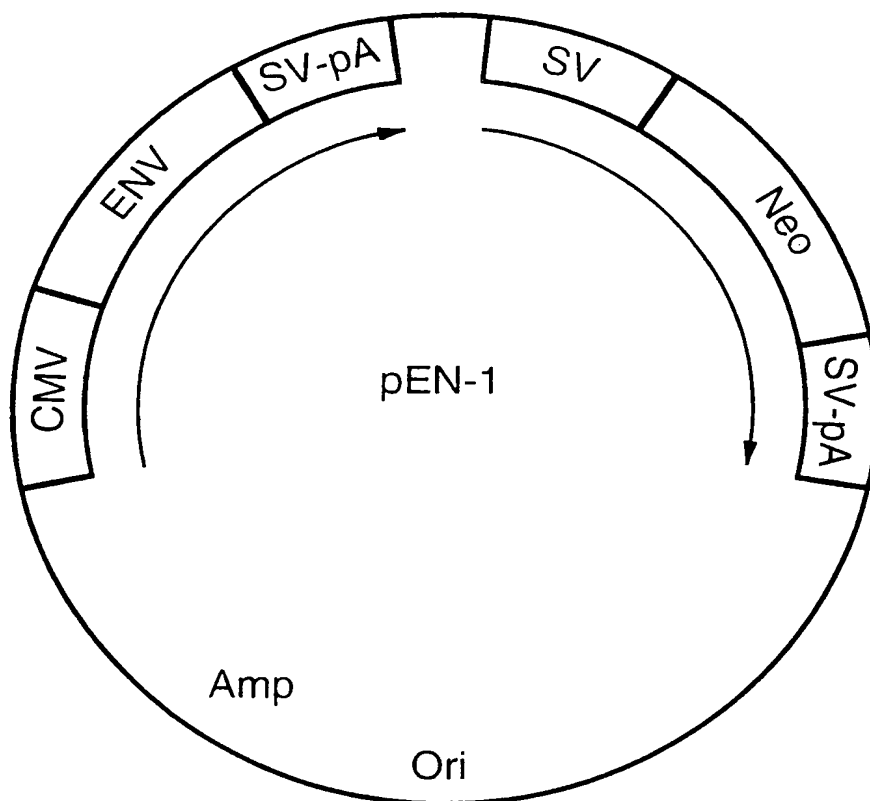


FIG.4

FIG. 5A

pCMV3-HPV1#1

0.491 UG/UL, 50UL

Construction: A synthetic mini-gene with the following sequence was cloned into the polylinker between SalI and EcoRI of the CMV3 vector resulting in pCMV3-HPV16. The gene encodes a protein consisting of five HPV16 T-cell epitopes (From NH2-to COOH terminus), E7: 49-57, 11-20, 82-90, 86-93, and E6: 29-38. Three alanines were introduced between the epitopes. The start codon is in bold letters, the Kozak sequence underlined, and the stop codons in bolded italic letters.

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Sell

TCGACGGCGCCACCATGAGAGCCATTACAATATTGTTACCTTGGCCGCCCTATATGTTA
GATTTCGAACGAGACAACCTGCAGCCGCTCTGTATAATGGCACACTAGGAATTGTGGCGG
CGGCGACACTAGCAATTGTGTGCCCCATCGCAGCAGCCACTATACATGATATATAATTAGA
ATGTGTGTAAATAGTACAATTCT

FCOR1

Translation of coding sequence:

	9	18	27	36	45	54											
ATG	AGA	GCC	CAT	TAC	AAT	ATT	GTT	AOC	TTT	GCC	GCC	TAT	ATG	TTA	GAT	TTG	
Met	Arg	Ala	Ala	Met	Asn	Ile	Val	Thr	Phe	Ala	Ala	Ala	Tyr	Met	Leu	Asp	Leu

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FIG.5A'

63	72	81	90	99	100
CAA CCA GAG ACA ACT GCA GCC GCT CTG TTA ATG GGC ACA CTA CGA ATT GTG GCC					
---	---	---	---	---	---
Gln Pro Glu Thr Thr Ala Ala Ala Leu Leu Met Gly Thr Leu Gly Ile Val Ala					
117	126	135	144	153	162
GCG GCG ACA CTA GGA ATT GTG TGC CCC ATC GCA GCA GCC ACT ATA CAT GAT ATA					
---	---	---	---	---	---
Ala Ala Thr Leu Gly Ile Val Cys Pro Ile Ala Ala Ala Thr Ile His Asp Ile					
171	180				
ATA TTA GAA TGT GTG TAA 3'					
---	---				
Ile Leu Glu Cys Val ***					

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FIG.5B

A Synthetic HPV Epitopes Mini Gene

49-57 E7

SalI Kozak Met (Arg Ala His Tyr Asn Ile Val Thr
 TCGACGCCGCCACCATGAGAGCCCATTACATATATTGTTACC
 GCGGCGGTGTTACTCTCGGTAATGTTATAACAATGG

III

I 11-20 E7

Phe)Ala Ala Ala (Tyr Met Leu Asp Leu Gin Pro Glu Thr Thr)
 TT'TGCCCGCCCTATATGTTAGATT'TGCCAACGAGACAACT
 AAACGCGCGGATATACAATCTAAACGTTGGTCTCTGTTGA

↑

↓

82-90 E7

II

Ala Ala Ala (Leu Leu Met Gly Thr Leu Gly Ile Val) **Ala Ala**
 GCAGCCGCTCTGTTAATGGGCACACTAGGAATTGTGCCCGCG
 CGTCGCGGAGACAATTACCCGTGTGATCCTTAACACCGCGCGC

VI

↑

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FIG. 5B'

86-93 E7

Ala(Thr Leu Gly Ile Val Cys Pro Ile) **Ala Ala** Ala(Thr Ile
 GCGACACTAGGAATTGTGTGCCCCATCGCAGCAGCCACTATA
 CGGTGTGATCCTTAAACACACGGGGTAGCGTCTCGGTGATAT

V

29-38 E6

His Asp Ile Ile Leu Glu Cys Val) * * *

CATGATATAATATTAGAATGTGTGTAATAGTGAG
 GTACTATATTATAATCTTACACACATTATCACTCTTAA RI

The mini-gene was assembled using five synthetic oligonucleotides (I-V, divided by arrows). The epitope sequences and the three alanine spacers are indicated. The start codon ATG is in bold letters; the Kozak sequence, underlined: the stop codons, in stars.

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pCMV3-HPVT#1

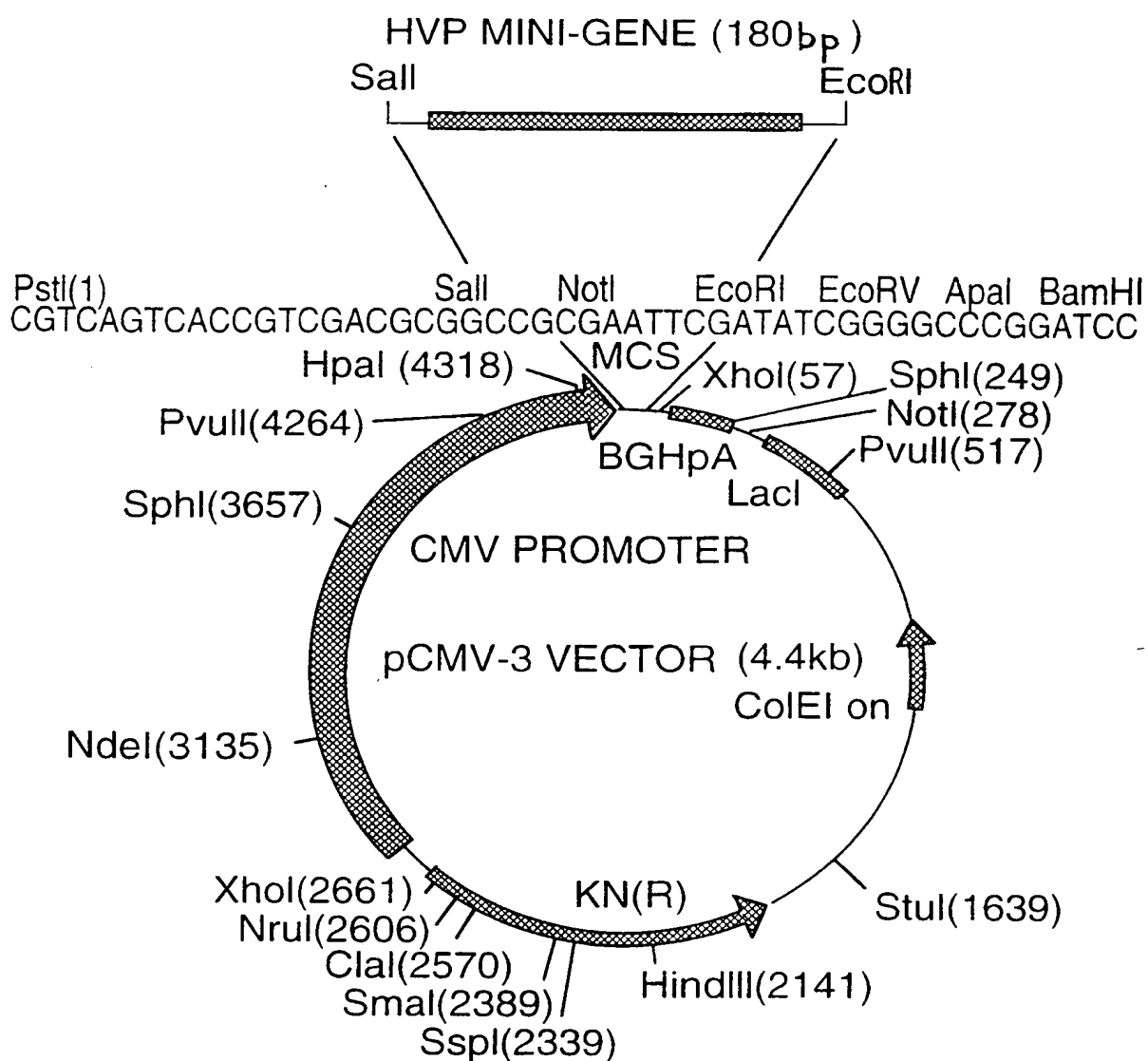


FIG.6